

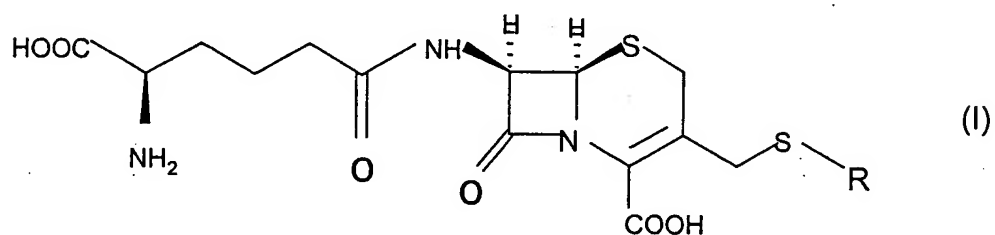
Claims

1. An enzymatic process for preparing 3-thiolated 7-aminocéphalosporanic acid derivatives comprising the steps:-

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enzymatically converting a 3-thiolated cephalosporin C of the formula I:

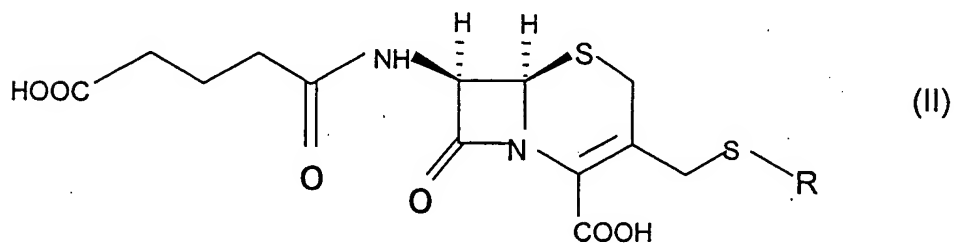
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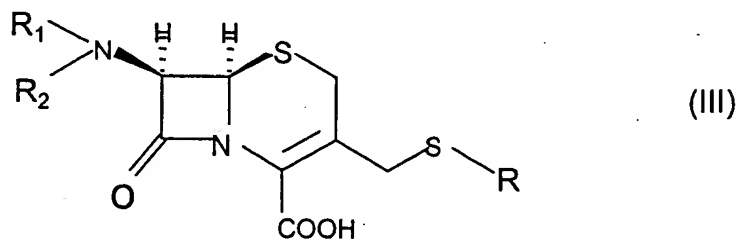
to form a 3-thiolated -glutaryl-7-ACA of the formula II

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and enzymatically converting a compound of formula II to form a 3-thiolated-7-ACA of the formula III

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wherein R is a heterocyclic group comprising at least one nitrogen atom and R₁ and R₂ are both hydrogen atoms or one of them is a hydrogen atom and the other is an acyl donor.

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2. A process as claimed in claim 1 wherein the 3-thiolated cephalosporin C of formula I is converted into a 3-thiolated-glutaryl-7-ACA of the formula II by:-

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reacting a compound of formula I with immobilised D-Amino acid oxidase in the presence of molecular oxygen;

separating the supported enzyme from the aqueous reaction mixture; and

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adding hydrogen peroxide to convert the 3-thiolated- α -ketoadipyl cephalosporanic acid into a compound of formula II.

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3. A process as claimed in claim 2 wherein the compound of formula I is reacted with immobilised D-Amino acid oxidase at a pressure of about 2 bar absolute, a pH of from 6.0 to 8.0, and a temperature of from 20°C to 30°C for a period of from 0.5 to 3 hours.

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4. A process as claimed in claim 2 including the step of washing the supported enzyme with a concentrated salt solution and adding hydrogen peroxide preferably in an amount equivalent to 30 to 50 ppm to the solution thus formed.

5. A process as claimed in claim 1 comprising the step of eliminating excess hydrogen peroxide from the solution, preferably by adding a catalyst to the solution.
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6. A process as claimed in claim 5 wherein the excess hydrogen peroxide is removed by adding catalase to the solution.
7. A process as claimed in claim 1 wherein a compound of formula II is converted into a compound of formula III by contacting a compound of formula II with immobilised glutaryl-7-ACA acylase.
10
8. A process as claimed in claim 7 wherein the reaction to form a compound of formula III from a compound of formula II is carried out at ambient pressure, at a pH of from 6.0 to 8.5 and at a temperature of from 20°C to 35°C, for a period of from 0.5 to 3 hours under an inert atmosphere.
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9. A process as claimed in claim 7 wherein the compound of formula III is precipitated by acidifying the reaction medium and the precipitate thus formed is subsequently washed and dried.
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10. A process as claimed in claim 1 wherein the enzymes are immobilised using a suitable cross-linker agent in a suitable solid support.
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11. A process as claimed in claim 10 wherein the enzymes are in the form of crystals of a size suitable for use as a biocatalyst.

12. A process as claimed in claim 1 wherein the enzymatic processes are carried out while maintaining the enzyme in dispersion in an aqueous substrate solution.
- 5
13. A process as claimed in claim 1 wherein the or each enzymatic process is carried out in a column.
14. A process as claimed in claim 1 including the step of recovering the enzyme for reuse.
- 10
15. A process as claimed in claim 1 wherein crystallisation of a compound of formula III is carried out at an acidic pH.
- 15
16. A process as claimed in claim 1 wherein the enzymatic conversion of a 3 thiolated cephalosporin C of the formula I to form a 3 thiolated-7-ACA of the formula III is carried out in one pot.
- 20
17. A process as claimed in claim 1 wherein R is a heterocyclic group comprising at least one nitrogen atom and optionally a sulphur or oxygen atom.
- 25
18. A process as claimed in claim 1 wherein R is a heterocyclic group selected from any one or more of the group comprising thienyl, diazolyl, tetrazolyl, thiazolyl, triazinyl, oxazolyl, oxadiazolyl, pyridyl, pirimidinyl, benzo thiazolyl, benzimidazolyl, benzoxazolyl, or any derivative thereof,

preferably 5-methyl-1,3,4-thiadiazol-2-yl, 1-methyl-1H-tetrazol-5-yl or 1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl.

- 5 19. A process as claimed in claim 1, wherein compounds of formula I are in a solid form or in the form of a non-toxic salt thereof.
20. A 3-thiolated-7-ACA of the formula III whenever prepared by a process as claimed in claim 1.
- 10 21. A process for the preparation of cephalosporin C antibiotics and derivatives thereof comprising forming a compound of formula III as defined in claim 1 and subsequent enzymation.
- 15 22. A process as claimed in claim 21 wherein the antibiotic is cefazolin, cefazedone, cefoperazone, cefamandol, cefatriazine, cefotiam or ceftriaxone.
- 20 23. An enzymatic process for preparing 3-thiolated 7-aminocephalosporanic acid derivatives as claimed in claim 1 comprising the steps:-

reacting cephalosporin C with a thiol compound of the general Formula IV

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R-SH

(IV)

wherein R is a heterocyclic group comprising at least one nitrogen atom,

to form a compound of formula I

and, after formation of the compound of formula I
removing excess thiol of Formula IV.

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24. A process as claimed in claim 23 wherein the excess thiol is removed by adsorption on an anion exchange resin.

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25. A process as claimed in claim 24 wherein the anion exchange resin is a microporous resin having a cross-linked acrylic copolymer structure.

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26. A process as claimed in claim 25 wherein the anion exchange resin comprises an 8% cross-linking containing functional thialkyl benzyl ammonium group.

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27. A process as claimed in claim 25 wherein the resin is in the chloride, hydroxy, phosphate or acetate cycle.

28. A process as claimed in claim 23 wherein the excess thiol is removed by crystallisation.

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29. A process as claimed in claim 28 wherein crystallisation is carried out at an acidic pH.

30. A process as claimed in claim 23 wherein the excess thiol is removed by crystallisation followed by adsorption on an anion exchange resin.

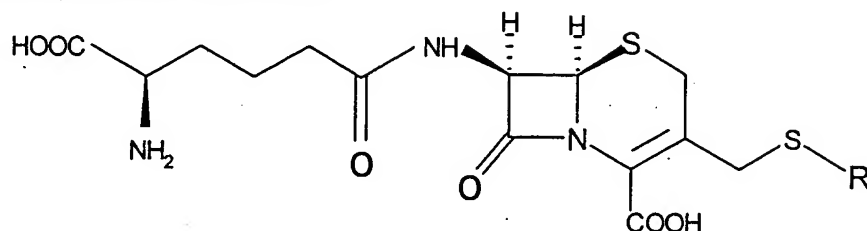
31. A process as claimed in claim 23 wherein the cephalosporin C is in an aqueous medium.
- 5 32. A process as claimed in claim 23 wherein the cephalosporin C is in the form of a concentrated cephalosporin C solution.
33. A process as claimed in claim 23 wherein the reaction is carried out at a pH of between 5.5 and 8.0, at a temperature of from 60°C to 80°C, for a period of from 1 to 12 hours.
- 10 34. A process as claimed in claim 33 wherein the reaction is carried out at a pH of approximately 6.0 and at a temperature of approximately 65°C.
- 15 35. A process as claimed in claim 23 wherein the thiol compound is present in an amount of between 1 and 5 mol/mol of cephalosporin C.
- 20 36. A process as claimed in claim 23 wherein R is a heterocyclic group comprising at least one nitrogen atom and optionally a sulphur or oxygen atom.
- 25 37. A process as claimed in claim 23 wherein R is a heterocyclic group selected from any one or more of thienyl, diazolyl, thiazolyl, tetrazolyl, thiadiazolyl, triazinyl, oxazolyl, oxadiazolyl, pyridyl, pirimidinyl, benzothiazolyl, benzimidazolyl, benzoxazolyl, or any derivative thereof, preferably 5-methyl-1,3,4-thiadiazol-2-yl, 1-methyl-tetrazol-5-yl or 1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl.

38. A process as claimed in claim 23 wherein compounds of Formula I are in a solid form or in the form of a non-toxic salt thereof.

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39. A compound of formula:-

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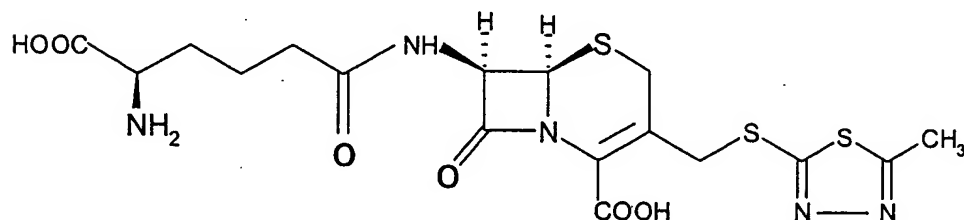
wherein R is a heterocyclic group comprising at least one nitrogen atom,

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obtained by a process as claimed in any preceding claim.

40. A compound of the formula:-

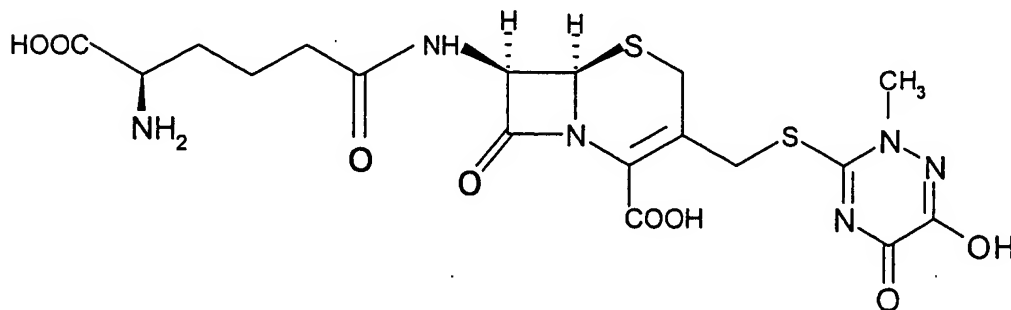
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wherein in formula I R is 5-methyl-1,3,4-thiadiazol-2-yl.

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41. A compound of the formula:-



5 wherein in formula I, R is 1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-
1,2,4-triazin-3-yl.

10 42. A process for the preparation of cephalosporin C antibiotics and
 derivatives thereof comprising forming a compound of formula I
 as defined in claim 1 and subsequent enzymation of the
 compound of formula I.

15 43. A process as claimed in claim 42 wherein the antibiotic is
 cefazolin, cefazedone, cefoperazone, cefamandol, cefatriazine,
 cefotiam and ceftriaxone.